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TITLE: Developing Breast Cancer Program at Xavier; Genomic and Proteomic Analysis of Signaling Pathways Involved in Xenohormone and MEK5 Regulation of Breast Cancer

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14. ABSTRACT
Xavier University (XU) and the Tulane Cancer Center (TCC) will build a core of human talent that will address scientific problems such as drug resistance and the effect of environmental agents on breast cancer (BC) in the African-American community. A multi-part research and training program will generate data, develop new research programs and train new faculty and African-American students in BC research. The first component will fund two research projects. The Wang and Burow project will elucidate a previously unexplored cellular signaling mechanism that leads to drug resistance in breast carcinoma cells derived from African American women and women of other ethnicities. The Wiese and Hill project will identify and characterize the genes and gene products associated with BC cell proliferation induced by exposure to pesticide mixtures and is relevant to the African American community in Southern States where pesticide exposure is relatively high. The second part of the program aims to increase the number of faculty at XU involved in BC research by supporting two junior faculty members to develop BC research projects with a TCC mentor. The third objective will support research training of XU undergraduates and pharmacy students. The fourth objective will provide workshops, seminars and research opportunities in BC research for the XU community. This program will enhance the understanding of unique aspects of BC development and progression among African American women and will contribute to the elimination of the "mortality gap" between African-American BC patients and women of other ethnicities.

15. SUBJECT TERMS
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Introduction

African American women are at higher risk for breast cancer (BC) mortality compared with their white counterparts. Over the past decade BC mortality has decreased 1%-2% per year in white women, but not in African-American women. The resulting "mortality gap" is a serious national problem, and understanding the reasons for it and developing solutions must be a high priority. Thus, BC research must focus on developing breast cancer models that would aim to accurately predict the disease development and progression among African-American women. ***We are convinced that increasing the involvement of African American students in BC research will greatly contribute to increasing awareness of the disease in the African American community, which in turn will increase the likelihood of early detection of the disease. Furthermore, the focus on the unique aspects of BC in African American women will lead to better understanding of the disease, and to better treatment options for African American women. This will eventually minimize or eliminate the BC "mortality gap".*** To this end we are developing a training program at Xavier University of Louisiana (XU) in collaboration with the Tulane University Cancer Center (TCC). More than 90% of Xavier's student body is African American has active programs (MBRS, MARC, RISE, NSF/MIE) designed to increase the number of minority students pursuing careers in medical and biomedical research. Through this BC training program, African American students will have the opportunity to become involved in BC research. Tulane (TU) and Xavier have a long history of collaborations involving joint centers and programs and individual collaborations between Tulane and Xavier faculty and staff are common. This new initiative will provide funds for yet another collaboration offering a unique opportunity for XU researchers to establish a BC research program for the benefit of XU students and, eventually, the African-American community. The goals of the training program are to create an environment that fosters BC research, in which XU investigators will receive substantive training and to complete substantive research projects of high relevance to the eradication of BC. The program will enable XU investigators to publish their results in peer-reviewed literature and advance toward independently funded BC research programs. The program includes two full research projects that involve an XU researcher and a qualified TCC mentor. The program will identify two additional XU researchers who have expressed an interest in BC research but do not have prior funding in BC. Participating XU faculty will get the opportunity to network and learn about BC research through participation in the TCC weekly seminar program and the signal transduction workshop that will focus on breast and prostate cancer. The two additional XU faculty involved will develop a mini-proposal in Y1-2 and carry out pilot studies with the advisory of a mentor faculty from TCC in Y2-4. The results of all program research studies will be used as a basis for future proposals in the area of BC. Yearly symposia will be held to provide information to XU students and faculty as well as to enrich the experience of the participating members regarding research opportunities in BC. Multiple project group meetings will be held each year to discuss current data, manuscripts in preparation, funding opportunities and issues regarding project operations.

Year Four Progress

The most significant factor in Y4 of this program was the return to normal for Xavier University after the continuing effects of Hurricane Katrina. Xavier University of Louisiana did an amazing recovery from the storm and after all buildings on campus were damaged by flood waters, reopened in January 2006. While no lab facilities involved in this program were directly impacted by flooding, all perishable supplies were lost. In addition, faculty involved in the program have been burdened with an increased teaching load after the evacuation and to make up for the lost time from the fall semester 2005, Xavier continued the fall semester in Jan-April 2005 and ran the Spring semester 2006 in the summer of 2006. Thus, time for research activities

was reduced in the programs Y2 and Y3. To make up for lost research time and thus save this program, we asked the DOD for a one year funded extension (Y5) in May of 2006 that was subsequently approved. In program year 4, Xavier teaching schedule and loads returned to normal and the ability to perform research also returned to normal with all labs involved in the program full operational and the summer months free from teaching.

The main goal of this program in Y4 was to get research programs back on track. It is important to note that by early 2007, the projects in this program (Wiese-Hill, Wang-Burow, Wolfgang-Miller) each had their research labs fully operational and research productivity back to at least where they were prior to Katrina. The personal and administrative task of bringing back these labs and maintaining these teams should not be underestimated. The combination of intense personal efforts by all involved from Xavier and Tulane combined with the immediate aid of a Katrina recovery grant from Tulane Cancer Center (Louisiana Cancer Research Consortium or LCRC) and the award of a funded extra year by the DOD has brought this program back together in a way that we expect that the original aims can be accomplished: Xavier will develop self sustaining cancer research programs well into the future.

Body

Task 1

Complete two substantive research projects of high relevance to eradication of breast cancer

Project 1

"Chemoresistance in Breast Carcinoma Cells: MEK5-BMK/Erk5 Expression and Proteomic Analyses"

Guangdi Wang, Ph.D., Department of Chemistry, Xavier University of Louisiana PI (Trainee)
Mathew E. Burow, Ph.D., Department of Medicine, Tulane University School of Medicine (Mentor)

Aim 1: To demonstrate the requirement for and the role of the MEK5 pathway in survival signaling and suppression of apoptosis in MCF-7 breast carcinoma cells.

(1). Implicate MEK5 activation in cell survival signaling, prevention of anti-estrogen and chemotherapeutic drug-induced cell death using MCF-7 stable, transiently transfected cells and ZR-75-30. (Months 1-18).
(2). Implicate apoptotic suppression as a mechanism for MEK5-mediated survival and drug-resistance (Months 12-24).

Year Four Progress (April 19, 2007-April 18, 2008)

In year four we have continued to pursue targets involved in MEK5-Erk5 mediated progression to an endocrine resistant and aggressive breast cancer cell phenotype. We describe that consistent with the EMT phenotype the MEk5 pathway drives breast cancer cells to an ER-negative phenotype. We further explore the role of rapid estrogen signaling in these cells. Our

data demonstrates that while the MEk5 cells exhibit a loss of genomic ERalpha expression and signaling they retain a rapid estrogen response characterized by PKC and Calcium signaling.

An NIH-R01 will be submitted in 2008:
"MEK5-Erk5 pathways in survival signaling and tumor progression to drug resistance"
(**Principal Investigator**) 25% effort
Agency: NIH-NCI (BMCT), Type: 1**R01CA**
The long-term goal of this project is to identify the signaling pathways critical to the development of resistance to chemotherapeutics agents and the progression to a hormone independent phenotype in carcinoma of the breast.

Results and Discussion:

MEK5-Erk5 signaling mediated progression of breast cancer cells to an ER-negative phenotype.

Our previous data suggests that higher levels of Erk5 activation are observed in ER-negative breast cancer cell lines. Additionally our data in the ER-positive MCF-7 line suggests that overexpression of CA-MEK5 drives these cells to a hormone-independent and ER-negative phenotype *in vivo*. We hypothesized that at some level the estrogen receptor pathways are affected by or subverted by MEK5-Erk5 signaling. To determine if activation of the MEK5-Erk5-Pathways affects ER expression levels we examined by PCR the expression of ER-alpha in the MCF7-VEC, MCF7-MEK5-(shRNA-empty) and MCF7-MEK5-Erk5-shRNA cells. These data show that in the MEK5 overexpressing cells, ER-alpha is downregulated at the mRNA and protein levels. Blockade of the MEK5-Erk5 pathways by shRNA-Erk5 restores ER-alpha expression at both the protein and mRNA levels. AP-2γ is a major factor in the regulation of ERα expression(162). A similar suppression of AP-2γ is also observed in the MEK5 cells suggesting a mechanism by which the MEK5 pathway may suppress ERα expression. Interestingly MEK5 expression stimulated an increase in expression of ERβ . To determine the effects of MEK5 on ER signaling we used an ERE-luciferase reporter assay. Parental MCF-7 cells transiently transfected with CA-MEK5 exhibited reduced ERE activation.

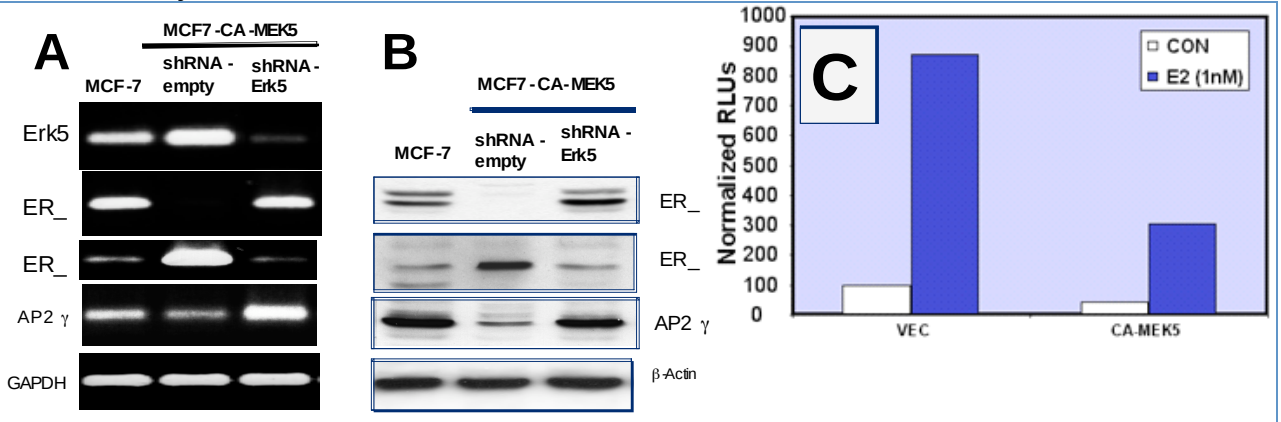


Figure 7. MEK5-Erk5 signaling suppresses ERalpha expression and activation. MCF7-VEC, MCF-7(CA-MEK5)-(shRNA-empty) and MCF-7(CA-MEK5)-(shRNA-Erk5) cells were grown in 5%CS-serum containing media to remove any endogenous estrogens from the experiment and harvested after 72 hours in estrogen free media. **(A),** Western blot analysis of ERalpha expression with beta-actin measured as a control. **(B)** RT-PCR analysis of ERalpha expression, Erk5 expression, or GADPH (control) expression. Data shown is representative analysis of three independent experiments. **(C)**, Parental MCF-7 cells were transiently transfected with ERE-luc (200ng/well) along with 300 ng/well of either vector or CA-MEK5 and treated 6 hours later with vehicle (DMSO) or 17β-estradiol (1 nM). Cells were harvested the next day for ERE-luciferase assay. Data is displayed as relative light units normalized to vehicle treated vector transfected cells (100) from a representative experiment.

Rapid estrogen signaling remains intact in the ERα-negative MEK5 breast cancer cells.

The above studies demonstrate that the MCF-7-MEK5 cells exhibit an ERα-negative phenotype and loss of ERE-activity and ER-mediated gene expression (PgR). Despite this these cells retain estrogen-responsive tumor growth and possess an Erk5-dependent upregulation of ERβ. Strong evidence now supports a role for rapid estrogen signaling in the proliferation and survival of both normal and neoplastic cells.

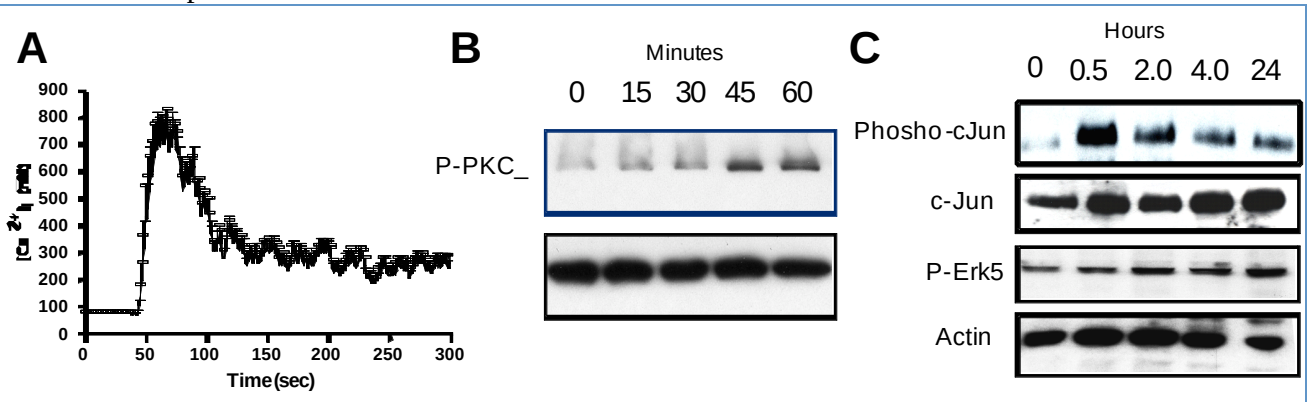


Figure 8. Estrogen promotes rapid calcium signaling and phosphorylation of PKC_ and c-JUN in MEK5 cells. **(A),** MCF-7-MEK5 cells were treated with estrogen and analyzed for intracellular calcium signaling. Individual Cells were analyzed for calcium resposne and data is represented as a average response for 13 individual measurements. **(B),** MCF-7-MEK5 cells were treated with estrogen (10 nM) for times indicated and harvested for Western blot analysis of phospho- or total –PKC_. Data is a representative experiemtn from four independent repeats. **(C),** MCF-7-MEK5 cells were treated for times indicated and harvested for Western blot analysis for phospho- or toal c-JUN, phospho-Erk5, and Actin levels.

While a number of pathways have been examined, our preliminary evidence demonstrates estrogen-mediated stimulation of a calcium and PKCα-dependent pathway exists in our ERα-negative MEK5 cells. Using MCF-7-MEK5 cells we demonstrate a rapid activation of both calcium and phospho-PKCα in response to estrogen stimulation. Estrogen stimulation also resulted in increased phosphorylation of c-Jun as well as Erk5 suggesting a link between rapid estrogen signaling and the MEK5-Erk5 pathway. While estrogen activation of PI3K-AKT and p38 has been reported in other cell systems we did not observe this in our MEK5 cells.

Aim 2: To characterize differences in protein expression between MCF-7N (APOP-Sensitive), MCF-7M (APOP-Resistant) and ZR-75-30 breast carcinoma cells and identify anti-apoptotic proteins, such as Survivin, within MEK5-expressing cell lines.

(1) Prepare samples for 2D gel separation. (Months 18-24).
(2) Separate proteins on 2D gel electrophoresis, compare differences in protein expression, and perform in-gel tryptic digestion of excised protein products. (Months 24-36).
(3) Sequences obtained from tryptic digests will be used to characterize and identify protein expression differences between drug resistant ZR-75-30 and MCF-7 breast carcinoma cells with a focus on known anti-apoptotic proteins or novel apoptotic domain containing proteins (BCl-2 homology (BH), baculovirus IAP repeat (BIR0, caspase activation recruitment domain (CARD), etc.). (Months 24-36).

Year Four Progress (April 19, 2007-April 18, 2008)

Overview
Close collaboration continued between Dr. Burow and Dr. Wang in their joint efforts to achieve the proposed research objectives. The major progress made in the past project year can be summarized as follows:

1. Comparative proteomics was carried out on the two breast cancer cell lines, MCF-7-MEK5 and MCF-7-VEC. As described earlier, MCF-7-MEK5 is a TNF-α-resistant breast cancer cell line derived from the wild-type, i.e. TNF-α-sensitive MCF-7 cells. MCF-7-MEK5 is characterized by 1) a morphological change consistent with epithelial-to-mesenchymal transition, 2) overexpression of MEK5 (MAPK), 3) resistant to tumor necrosis factor-α and tamoxifen, and 4) estrogen independent. MCF-7-VEC represents a control cell line transfected with empty vector.
2. We have identified seven protein spots that are differentially expressed in MCF-7-MEK5 and MCF-7-VEC using 2-D gel electrophoresis. Real time PCR analysis confirmed the findings in proteomics work.
3. We have obtained evidence at both the gene and protein expression level, that MCF-7-MEK5 cells express EMT markers, suggesting involvement of MEK5 in the regulation of EMT in breast cancer cells.
4. A manuscript based on these findings has been submitted to the *Journal of Breast Cancer Research* in March of 2008.
5. A proposal for the DoD IDEA AWARD (oppW81XWH-08-BCRP-IDEA) is in preperation for submition in May, 2008. The title: MEK5-Erk5 Signaling Regulates Epithelial-to-mesenchymal Transition in Breast Cancer Progression.

Results and Discussion:

As shown in **Figure 1**, a total of seven protein spots were identified that differed significantly in the two cell lines. These protein spots yielded rich peptide fragments, and were found to have similar theoretical and experimental molecular weights (MW) and isoelectric points (pI). The seven protein spots were KRT19, GSTM3, VIM, HSPA4, GSTP1, CKB and KRT8. In MCF-7-MEK5 cells, VIM, HSPA4, GSTP1 and CKB were upregulated while KRT8, KRT19, and GSTM3 were downregulated as compared to MCF-7-VEC cells. Indeed, expression of KRT8 in MCF-7-MEK5 cells was below detection limit under the current proteomics conditions.

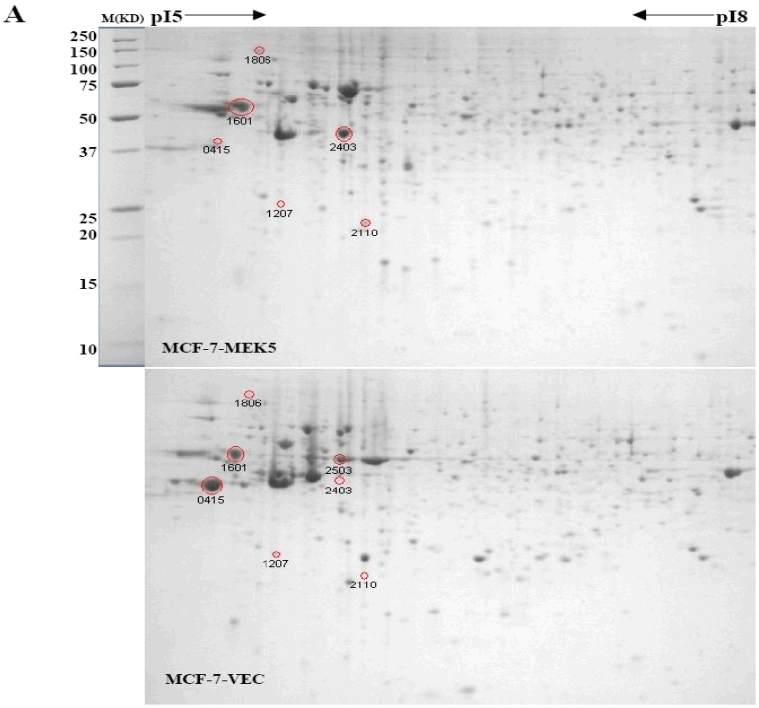


Figure 1. 2-DE images of MCF-7-MEK5 and MCF-7-VEC cells. **A**. 2-DE image of the total proteins extracted from MCF-7-MEK5 and MCF-7-VEC cells. **B**. A total of seven differentially expressed protein spots were identified, and the changes of protein expressions are shown in **Table 1**.

Figure 2. The differences of genes vim, krt8, krt19, hspa4, gstp1, gstm3, and ckb in MCF-7-MEK5 and MCF-7-VEC cells. **A**. Regular RT-PCR assay. **B**. Real-time PCR assay, the normalized expression folds in MCF-7-MEK5 cells were 814.15±145.23 (vim), 0.01±0.00 (krt8), 0.00±0.00 (krt19, undetected), 1.51±0.56 (hapa4), 40637.45±15815.03 (gstp1), 0.13±0.01(gstm3), 38.59±7.87 (ckb) compared to MCF-7-VEC cells. The gene actb was used as an internal control.

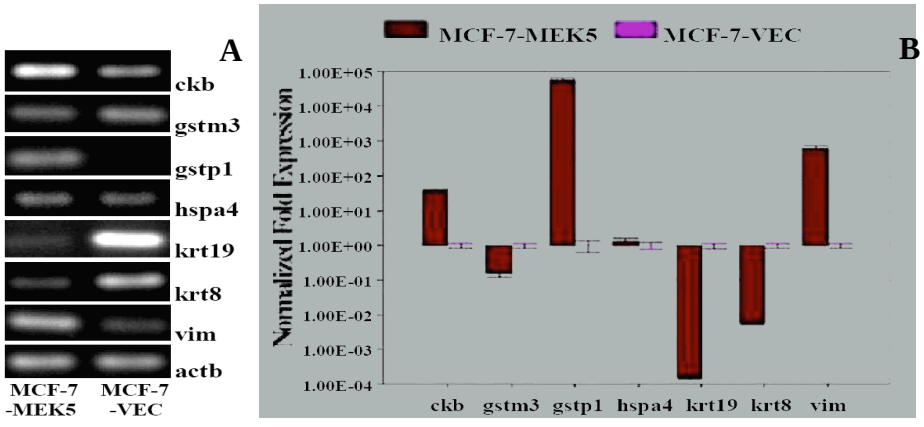


Table 1 Identification of the seven protein spots with LC/MS

Spot No	Protein Symbol	Theoretical MW (KD)	Experimental MW (KD)	Theoretical pI	Experimental pI vs MCF-7-VEC)	Ratio (MCF-7-MEK5
0415	Keratin 19 (KRT19)	44.07	46	4.9	5.2	0.01
1207	Glutathione S-transferase Mu 3 (GSTM3)	26.54	26	5.25	5.7	0.37
1601	Vimentin (VIM)	53.62	55	4.91	5.2	2.14
1806	Heat shock 70 kDa protein 4 (HSPA4)	94.24	100	5.03	5.3	1.81
2110	Glutathione S-transferase P (GSTP1)	23.34	23	5.32	5.8	5.41
2403	Creatine kinase B-type (CKB)	42.62	45	5.25	5.7	57.67
2503	Keratin 8 (KRT8)	53.67	50	5.38	5.8	0

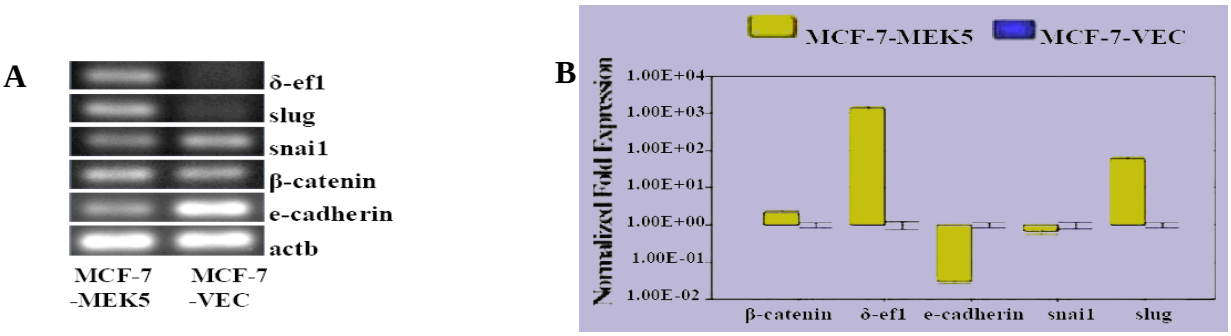


Figure 3. The differences of genes involved in EMT in MCF-7-MEK5 and MCF-7-VEC cells. **A**. With regular RT-PCR assay, the genes slug, δ-ef1, and β-catenin were upregulated, while e-cadherin and snai1 were downregulated in MCF-7-MEK5 cells compared to MCF-7-VEC cells. **B**. With real-time PCR, the normalized gene expression folds in MCF-7-MEK5 cells were 0.03±0.00 (e-cadherin), 2.16±0.27 (β-catenin), 0.68±0.11 (snai1), 60.79±7.94 (slug) and 1369.18±164.96 (δ-ef1) compared to MCF-7-VEC cells. The gene actb was used as an internal control.

These proteins are known to have various roles in cellular processes including detoxification, proliferation, metabolism, and cytoskeletal organization. At least one of these proteins is also implicated in EMT, suggesting a possible connection between TNF-α resistance and EMT.

It is well known that human cytosolic GSTs, which play an important role in cellular detoxification pathways, have a high polymorphism and are divided into six classes: α, μ, π, ω, θ and ζ [1]. Although GSTs have been implicated in many drug-resistance tumors [2, 3], the effects of each class or sub-class of GSTs on drug resistance are still not clear. In most instances, GSTP1 (π) is believed to contribute to drug resistance via direct detoxification, and has an overexpression in the established breast cancer cells resistant to some chemotherapeutic drugs such as adriamycin [4-6]. Transfection or inhibition of GSTP1 can result in retaining or losing the drug resistance in some established cancer cells [7]. Our study verified the overexpression of GSTP1 at both the gene and protein levels in the established TNF-α-resistant breast cancer cell line (MCF-7-MEK5). These results suggest the involvement of a similar detoxification pathway in TNF-α-resistant MCF-7 cells. Interestingly, GSTM3, the sub-class of GSTM (μ), was found to be downregulated in TNF-α-resistant MCF-7 cells. In fact, the effects of GSTM in anticancer

drug resistance have been controversial. For example, the overexpression of GSTM was observed in mitoxantrone-resistant MCF-7 cells upon induction of prodigiosin [8] but not in MCF-7 cells resistant to adriamycin [6]. Thus, the expression of GSTM appears to be dependent upon mechanisms underlying different anti-cancer drug resistance.

HSPA4 (also known as APG-2 or HSP70RY) is not a member of HSP70 family, but an HSP110 homologue [9, 10]. HSPA4 is overexpressed in hepatocellular carcinoma and is thought to play a role in cell proliferation [11]. It binds to the tight junction protein OZ-1 to regulate the transcriptional activity of ZONAB, which is associated with the transcription of the oncogene erbB-2 [12]. There have been scarce reports on the involvement of HSPA4 or APG-2 in drug resistance in tumors. Our study indicated that HSPA4 was overexpressed in MCF-7 cells resistant to TNF-α on both gene and protein levels. Such overexpression of HSPA4 may be related to the development of TNF-α resistance in MCF-7 breast cancer cells. Additionally, a metabolic factor, CKB, was found to be overexpressed in MCF-7-MEK5 cells, suggesting its possible involvement in the changes of cellular phenotypes in TNF-α resistance mediated by MEK5/ERK5 signaling.

Finally, both VIM and KRT belong to intermediate filaments (IFs), which are considered the principle cytoskeletal proteins in mammalian cells [13]. It is believed that the overexpression of VIM, which is related to poor prognosis in breast cancer patients presenting metastasis potential [14,15], results in a more invasive capacity of breast cancer cells *in vitro* and *in vivo* [16-18]. Kokkinos and his colleagues proposed that the IF transformed from a KRT-rich to a VIM-rich network in the process of EMT in cancers [16]. Previous studies have shown [19] that MCF-7 breast cancer cells do not typically express VIM but exhibit strong expression of KRT; the acquisition of VIM expression and the loss of KRT19 expression were associated with adriamycin-resistant MCF-7 cells compared to their parental cells. Our results showed increased VIM expression and decreased KRT19 and KRT8 expression in TNF-α-resistant MCF-7 cells. These findings, consistent with the cytoskeletal reorganization seen in both EMT and drug resistance, provoked further evaluation of the EMT markers snail, slug, δ-ef1, β-catenin and e-cadherin in the TNF-α-resistant MCF-7 cells.

The profound morphological changes and enhanced invasive capabilities of EMT in various cancers are thought to be regulated by several transcription factors, including Snail, Slug and δ-EF1. Increased expressions of Snail and Slug have been reported in invasive breast tumors compared to noninvasive ones, and are associated with lymph node metastases [20]. Increased levels of δ-EF1, also seen in invasive breast tumors, have been correlated with dedifferentiation [21]. These transcription factors, known to regulate EMT in development, have all been shown to repress E-cadherin, the primary cell adhesion molecule in epithelial tissue [21-25]. The loss of epithelial cell-cell adhesion through decreased expression of E-cadherin is the hallmark of EMT that permits acquisition of a motile phenotype [26]. Interestingly, the gene snai1 in MCF-7-MEK5 cells was not upregulated in spite of the downregulation of the gene e-cadherin in our study. It appears that the roles of snai1 and slug, which belong to the same zinc-finger protein superfamily, are not identical in MCF-7-MEK5 cells resistant to TNF-α.

The downregulation of E-cadherin, which serves as both a tumor suppressor and an invasion repressor, can lead to the loss or dislocation of β-catenin, in addition to loss of cell adhesion [27]. Several studies have shown that this dislocation of β-catenin from E-cadherin leads to nuclear localization of β-catenin where it acts as a cofactor in the transcription of other regulators of EMT in breast cancer [28, 29]. While it is unclear whether this also occurs through upregulation

of the β-catenin gene in EMT, our results showed increased β-catenin expression in MCF-7-MEK5 cells resistant to TNF-α.

In conclusion, seven differentially expressed proteins have been identified by proteome and gene analyses, suggesting that upregulation of VIM/vim, HSPA4/hspa4, GSTP1/gstp1, and CKB/ckb, and downregulation of KRT8/krt8, KRT19/krt19 and GSTM3/gstm3 are related to MEK5/ERK5-mediated TNF-α resistance in an established MCF-7 cell line. Further gene analyses of this cell line indicated expression of an EMT phenotype, suggesting an association between EMT and MEK5/ERK5-mediated TNF-α resistance. Additional studies are needed to clarify the functions and involvement of these proteins in the mechanisms of MEK5/ERK5-mediated TNF-α resistance and EMT in human breast cancers.

Aim 3: In this task we will use RNA interference strategies to validate a role for the Erk5 pathway in downstream gene expression and in suppression of chemotherapeutic drug-induced apoptosis. Our preliminary analysis revealed survivin expression was increased in drug-resistance and MEK5 expressing breast carcinoma cells. Subsequently we will characterize the role of these downstream targets such as Survivin, in suppression of apoptosis and drug-resistance.

(1) Optimize pSUPER base RNA interference (RNAi) suppression of ERK5 expression in breast carcinoma cells (Month 15-18).
(2) Confirm a role for Erk5 signaling in MCF-7N-CA-MEK5, and MCF-7M-(RESIST) cell survival using pSUPER-Erk5-RNAi. (Months 18-28).
(3) Develop/validate RNAi strategies for Survivin suppression using pSUPER method as above. Use RNAi to implicate Survivin expression in drug resistance and apoptotic signaling of MCF-7 and ZR-75 breast carcinoma cells (Months 24-36).
(4) Develop, validate and use RNAi strategies for novel targets identified from proteomic analysis of drug resistant breast carcinoma cells from Aim 2. (Months 36-48).

No Progress on this Aim in Year 4

Project 2
Interactions of estrogen and progestin active environmental chemicals on BC cell proliferation, survival and gene expression

Thomas E. Wiese, Xavier University College of Pharmacy PI (Trainee)
Steven R. Hill, Tulane University School of Medicine (Mentor).

Year Four Progress (April 19, 2007-April 18, 2008)

Research Assistant
Mr. H. Chris Segar continues as research assistant on this project.

Collaboration between Dr. Wiese at Tulane Cancer Center and Dr. Hill
Dr. Wiese has been in close contact with Dr. Hill since the start of this project through phone, email or meetings. While the project was designed to take place entirely in the Wiese lab, Dr. Hill continues to provide input on experimental design and data interpretation. The main contribution of Dr. Hill to this project has been discussions relating to the use of microarray technology to identify specific genes or classes of genes that may be related to the observed

mixture effects (see Y2 progress of Aim 2 below). Dr. Hill has also provided insight regarding the management of the overall training program (see tasks 2 and 3 below).

Preliminary and Y1-Y3 results summary

The series of pesticides included in this study included isomers and metabolites of DDT and methoxychlor. Each are known to have weak estrogen, androgen and/or progesterone activity. A series of MCF-7 proliferation studies were conducted to identify novel interaction effects of binary mixtures of these compounds. The initial studies were designed to include one pesticide at the lowest observed effect level (LOEL) and the other at the highest dose possible (10-5 M). Experiments were also conducted to determine if mixing the pesticide (high dose) with sub-optimal concentrations of estradiol-17b (E2) enhanced estrogen induced proliferation. This series of experiments did not identify mixture combinations with more than the additive cell proliferation activity expected from the compounds alone at the same concentrations. These same mixtures were examined in the MVLN estrogen responsive reporter gene assay where similar additive effects were also observed. See Y1 progress report for more information. At this point, Dr. Wiese decided to examine mixtures that contained one of the organochlorine pesticides along with one of three organophosphate pesticides. We have observed a positive sensitizing or potentiation effect of organophosphate pesticides on the weak estrogen dependant proliferation activity of organochlorine pesticides (increased potency). This action can be eliminated by antiestrogen and is likely estrogen receptor (ER) dependant. The observation that this sensitizing effect was not observed in the reporter gene system suggests that the mechanisms involved are more complex than a simple stimulation of classical ER transactivation activity. Finally, the observation of this sensitizing effect suggests a hypothesis that exposure to low levels of weakly estrogenic pesticides in combination with an organophosphate pesticide might result in more breast cancer cell proliferation than would be expected by the organochlorine alone. The organophosphate compounds in this study are known to have antiandrogen activity. Considering that androgen agonists are known to inhibit estrogen regulated processes in some cells, it is reasonable that treatment with antiandrogens may relieve such suppression, resulting in a relative increase in organochlorine induced estrogen activity. The organochlorine compounds in the study are considered persistent contaminants with long elimination half lives. Thus, chronic exposure to low concentrations may have more estrogenic activity than would be expected if cells are sensitized or stimulated by periodic exposure to organophosphate pesticides. Contamination from older pesticides that are no longer used might be more significant if one is exposed to current use pesticides. See Y1 - Y3 progress reports for more information.

Aim 1: Examine the effects of binary mixtures of estrogen and progestin active environmental compounds on cell proliferation and survival.

(a). Develop treatment mixture matrix and plan for proliferation experiments (Months 1−2).

(b). Perform cell proliferation studies with binary mixtures of pesticides (Months 1−18

(c). Identify mixtures with novel effects on cell proliferation (Months 6−20).

In Year 3, we re-established the lab after Katrina and repeated experiments used to obtain the above results. This was an effort to validate that the new cell, new reagents and new lab conditions were able to produce the same results as obtained prior to Katrina. These experiments did correlate with previous findings.

Mixture combinations that produced the most dramatic sensitization effect in the breast cancer

proliferation assay were selected for PCR array analysis in Aim 2. These are: Parathion and opDDT, Fenitrothion and opDDT, and HPTE and opDDT.

Year Four Progress (April 19, 2007-April 18, 2008)

No new data was generated in Y4 for this aim.

Aim 2: Conduct cDNA microarrays to define a set of genes that are coordinately or differentially regulated by treatment with environmental hormones. Preparations from cells grown and exposed to mixtures of hormone active pesticides in the Wiese Lab will be evaluated for differential expression of genes in the Tulane Center for Gene Therapy.

(a). Identify target genes related to breast cancer cell proliferation from literature searches that will be used in gene array studies (Months 1−12).

(b) Prepare cells for gene array analysis after exposure to mixtures of pesticides. (Months 9−24).

(c) Run gene array analysis on cell preparations and analyze data (Months 12−36).

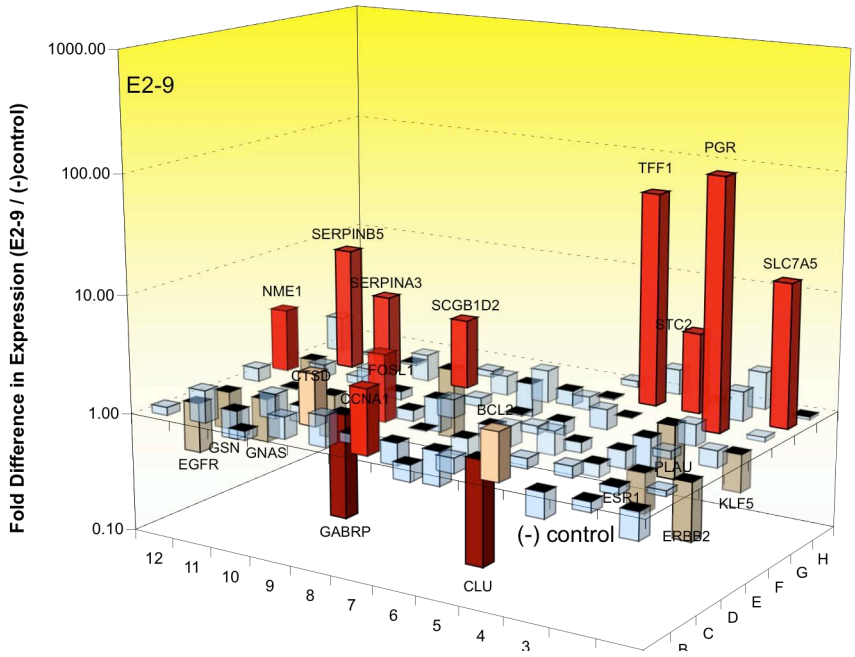
In Year 3 we obtained the equipment and prepared the lab for the genomic phase of this project. To obtain the equipment, Real Time PCR, a proposal was submitted to the Xavier College of Pharmacy and a BioRad iQ5 was purchased for use in the Wiese Lab for this project and subsequent use in Pharmacy teaching labs. The support of the college of provide this $36,000 instrument is significant. In addition, considerable time was spent testing RNA prep methods and developing 6 well plate cell seeding methods that would produce at least 10 ugms RNA. The Qiangen RNA prep method with the shredder and DNA removal was found to work well.

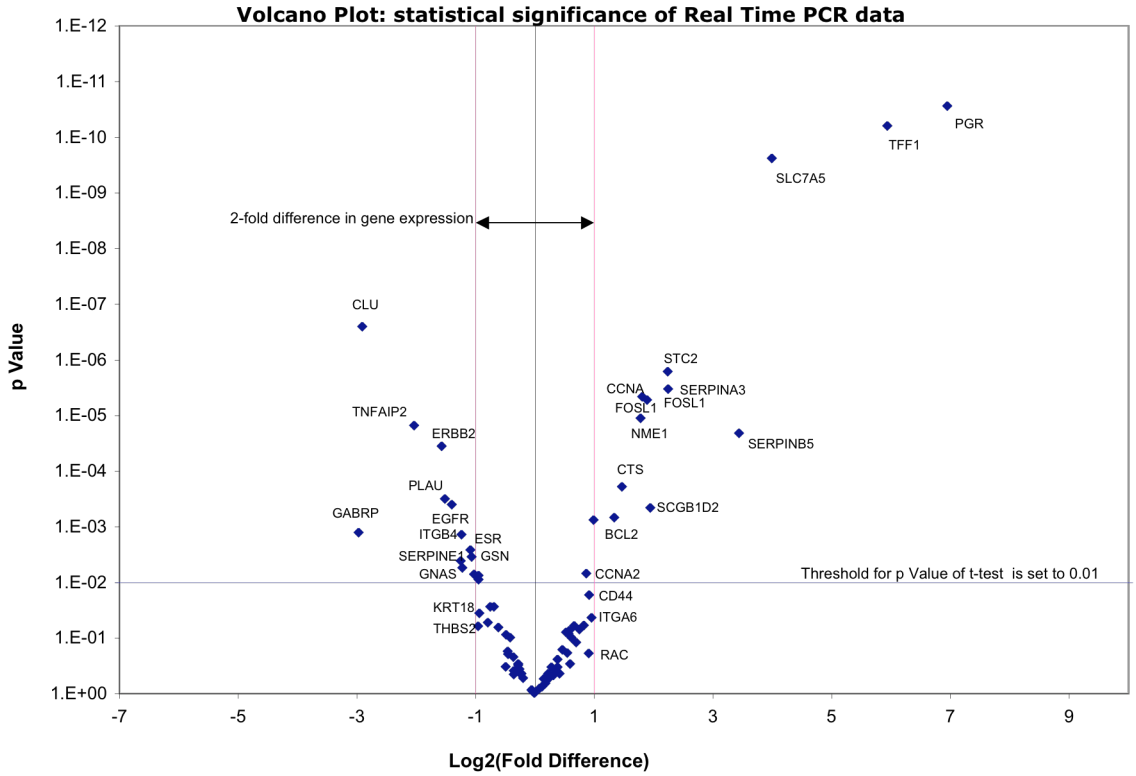
Year Four Progress (April 19, 2007-April 18, 2008)

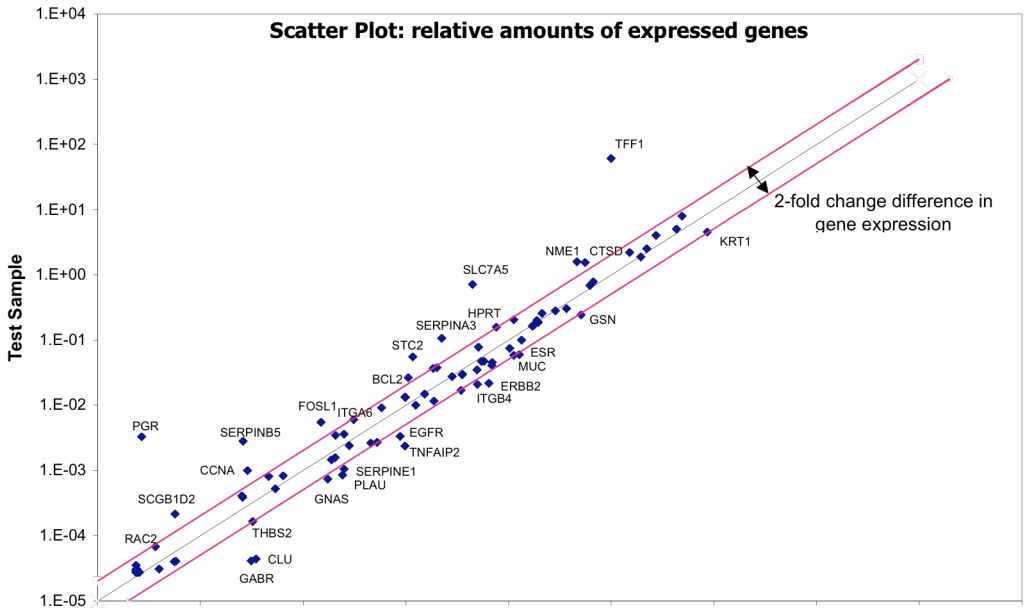
In Y4, we have completed training and application development of the PCR Array assay. Methods have been optimized to obtain pure RNA and the actually PCR Array method has been optimized and validated for precision. The technical aspects of this assay were more complex than anticipated and this process took more time than expected. However, we now have confidence that the procedure from plating and treating cells, harvesting RNA, making cDNA, loading and running PCR array plates and preliminary analysis of multiple experiments has all been worked out. Preliminary data from the PCR array analysis will be presented at the Era of Hope meeting in June 2008. Results from preliminary data suggests that there are unique gene expression profiles for the pesticide mixture treatments in MCF-7 cells in relation to the untreated and treatment with estradiol. Some PCR array singe run results for and statistical analysis of multiple runs of controls (5 replicates) are shown below in 3D bar graphs and volcano s well as scatter plots. It is important to note that typical estrogen regulated genes are shown to be significantly induced by the estradiol treatment in relation to the untreated cells and that the precision of the assay over multiple runs is high (p value 0.01). Also shown below is a preliminary plot of gene fingerprints (expression fold over control) for the pesticides and mixtures in this study.

The primary goal of Y5 is to complete the PCR array analysis of all of the pesticide mixtures evaluated in Aim #1 and then to perform data analysis to determine what genes are unique to each treatment.

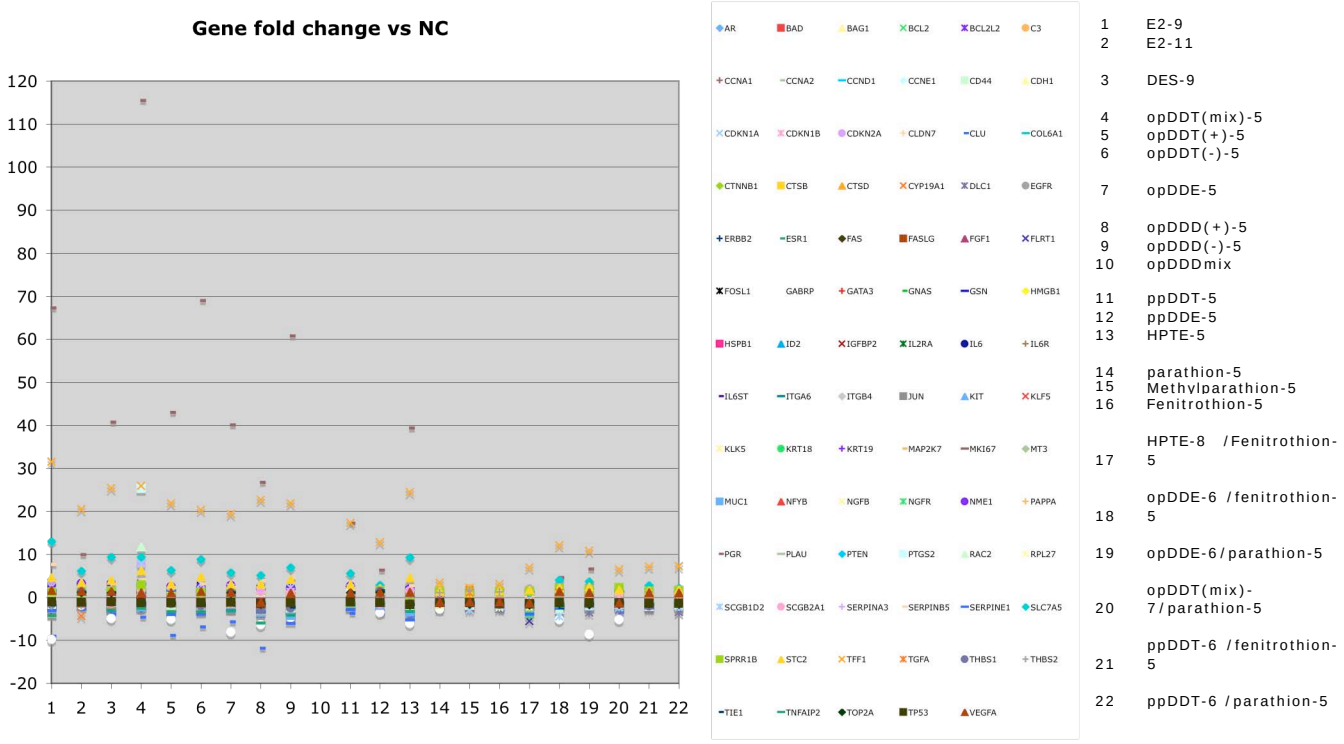
MCF-7 Cells: E_2 10^{-9} M vs Blank







Pesticide Gene Expression Fingerprints



Aim 3: Confirm the expression pattern of genes identified by microarray through analysis of gene products (mRNA or protein).

(a) Select 6−10 genes that have been shown by differential display to have novel expression patterns as a result of pesticide mixture treatment (Months 14−36).

(b) Obtain probes for Northern blot analysis of selected genes (Months 14−36).
(c) Perform Northern blots to confirm expression observed in micro array studies (Months 24−48).
(d) Obtain antibodies for Western blot analysis of selected genes (Months 14−36).
(e) Perform Western blots to confirm expression observed in micro array studies (Months 24−48).

Year Four Progress (April 19, 2007-April 18, 2008)
No progress on this aim in year 4. We must complete Aim 2 first.

Deliverables/measurable outcomes:
Drs. Wang and Wiese will prepare or oversee the following:

1. Semiannual reports will be submitted to the PI.

Year Four Progress (April 19, 2007-April 18, 2008)
These reports were submitted and have bee used to make this progress report.

2. Students involved in the research will present a poster at the annual research workshop (Months 12, 24, 36, 48).

Year Four Progress (April 19, 2007-April 18, 2008)
Drs. Wiese and Wang have had no students to working on their projects in Y4. Dr. Wolfgang (see Task 2a below) has had three students working with him on his new project: Hue Danh, Thy Ho-Pham, Vi Tran. It should be noted that all Xavier students involved in the DOD programs at Xavier present at the universities annual Festival of Scholars in April.

3. One competitive grant application will be submitted by the end of the funding period.

Year Four Progress (April 19, 2007-April 18, 2008)
Two grants will be submitted by Project #1 team in Y5:

"MEK5-Erk5 pathways in survival signaling and tumor progression to drug resistance"
(**Principal Investigator**) 25% effort
Agency: NIH-NCI (BMCT), Type: 1**R01**, 07/01/09-06/30/14,
The long-term goal of this project is to identify the signaling pathways critical to the development of resistance to chemotherapeutics agents and the progression to a hormone independent phenotype in carcinoma of the breast.

A proposal for the DoD IDEA AWARD (oppW81XWH-08-BCRP-IDEA) is in preperation for submition in May, 2008. The title: MEK5-Erk5 Signaling Regulates Epithelial-to-mesenchymal Transition in Breast Cancer Progression.

4. Papers will be submitted to peer reviewed journals through the funding period.

Year Four Progress (April 19, 2007-April 18, 2008)
A manuscript has been submitted by Project #1 to the *Journal of Breast Cancer Research* in March of 2008:

"Proteomic analysis of tumor necrosis factor-alpha resistant human breast cancer cells reveals a MEK5/Erk5-mediated epithelial-mesenchymal transition phenotype"
Changhua Zhou, Ashley M Nitschke, Wei Xiong, Qiang Zhang, Yan Tang, Michael Bloch, Steven Elliott, Yun Zhu, Lindsey Bazzone, David Yu, Christopher B Weldon, John A McLachlan, Rachel Schiff, Barbara S Beckman, Thomas Wiese, Kenneth P Nephew, Bin Shan, Matthew Burow and Guangdi Wang

Training deliverables:

1. The Tulane Cancer Center in conjunction with the Section of Hematology and Medical Oncology and The Cell Signaling group will be directly involved in providing breast cancer research training for Xavier Investigators.

Year Four Progress (April 19, 2007-April 18, 2008)

The support provided from the TCC to each project is described within the progress reports of each project above. In addition, TCC support for the program as a whole is detailed in Task 3 below.

2. Toward the end of the project period, Drs. Wang and Wiese will be Co−PIs in writing an R01 grant in collaboration with Drs. Burow and Hill.

Year Four Progress (April 19, 2007-April 18, 2008)

While no R01 collaborative grants are in preparation at this time, Dr. Burow (Tulane) from Project #1 will submitt an R01 grant related to this project and, Project #1 team (Drs. Wang and Burow) will submit a DOD Idea award in May 2008 (see Aim 3, Part 3 above).

Task 2

Assist two Xavier junior faculty to become more competitive in breast cancer research

a. Identify two Junior Faculty with interest in breast cancer research (Month 1).

Year Four Progress (April 19, 2007-April 18, 2008)

Dr. David Wolfgang, XU Chemistry, has developed a project with Dr. Charles Miller of the TCC (see below). Dr. Wiese is assisting Dr. Syed Muniruzzaman, XU Biology to develop a project involving rare sugars as potential cancer chemotherapeutic agents.

b. Establish participation of the selected Junior Faculty in Tulane Cancer Center seminars and the weekly signal transduction workshop focused on breast and prostate cancer (Month 2).

Year Four Progress (April 19, 2007-April 18, 2008)

All faculty involved in the Xavier DOD Cancer programs are now integrated into the Molecular Signaling focal group of the LCRC. These faculty are exposed to a wide range of cancer research by attending the Molecular Signaling research meetings and these faculty are now building stronger ties between Xavier and the LCRC. We have established a twice monthly Cancer Research lunch meeting at Xavier (see below).

c. Determine Tulane Cancer Center mentors for the Junior Faculty and submit a two−page mini proposal for review of the PI and alternate PI (Month 6).

Year Four Progress (April 19, 2007-April 18, 2008)

Junior Faculty Project #1
Dr. David Wolfgang (XU Chemistry) has developed a project with Dr. Miller at the TCC and progress is reported below.

Note: In Fall 2007, Dr. Wolfgang was not approved for tenure at Xavier. He has accepted a terminal contract ending May 2009. Considering that he has mad significant progress on his project and that he continues to involve Xavier students in his research, The PI has elected to continue supporting Dr. Wolfgang in this program through the spring of 2009.

The Effect of Cellular Levels of the Hsp90 Co-chaperon p23 on the Stress Response of Mice Fibroblasts.

Original Specific Aims:

1) To determine if the amount of p23 in mouse fibroblasts affects the toxicity of anti-tumor compounds geldanamycin and herbimycin A.

2) To determine if the amount of p23 in mouse fibroblasts affects the toxicity of compounds (cadmium and arsenate) known to initiate the heat shock response.

New Specific Aim:
To produce a peptide that will bind to p23 and inhibit the binding of p23 to Hsp90, making the cells functionally p23 null.

Introduction:
Hsp90 is a chaperon protein that plays a role in the maintenance of steroid hormone receptors in their high affinity form. Hsp90 also interacts with kinases and polymerases. Proper Hsp90 function requires additional factors (co-chaperones) such as p23. Hsp90 has been linked to proteins involved in all six features found in almost all cancers: 1) self sufficient growth signaling, 2) insensitive to signals that halt the cell cycle, 3) evade apoptosis, 4) angiogenesis, 5) metastasis, and 6) unlimited potential for cell division. As such, the understanding of Hsp90 and its co-chaperones is vital to understanding and treating cancer. The protein p23 has been shown to maintain Hsp90 in its active (ATP bound) form. p23 is also part of the Hsp90 complex that is involved in chaperoning estrogen receptor alpha. The fact that p23 is up-regulated in cancer cells suggests that it may play a role in tumor growth. It has also recently been shown that overexpression of p23 in MCF-7 cells enhances adhesion and invasion. Dr. Charles Miller has mice that are heterozygous for the p23 gene, one copy has been knocked-out. We obtained cells from embryos since mice that are p23 homozygous null are not viable beyond birth. After determining whether or not the presence of p23 plays a role in tumor growth, a peptide will be synthesized to hopefully disrupt the binding of the Hsp90-p23 chaperone complex.

Methods:
Mice heterozygous for the p23 gene are mated and females are sacrificed about a day before birth. Skin cells are plated in T25 flasks with DMEM media supplemented with 10% Fetal

Bovune Serum, L-glutamine, sodium pyruvate, and antibiotics. When the plates are confluent, the cells are treated with trypsin to remove them from the flask, counted, diluted to 12,000 cells per mL, and seeded in 96-well plates at a concentration of 1200 cells per well. These cells are grown overnight at 37ºC. Doses of the test compound are diluted in DMEM media supplemented as described. Geldanamycin and Herbimycin are tested in the range of 10 µM to 1 nM final concentration. Cadmium and arsenate were tested in the range of 0.3 to 10 µM final concentration. The old media is suctioned out of the wells and replaced with 100 µL of media with the appropriate dose. Three sets of controls and prepared; wells with cells and "dosed" with media only function as a positive control, wells without any cells function as a negative control, and cells "dosed" with media and DMSO control for the fact that Geldanamycin and Herbimycin A are diluted from a stock solution dissolved in DMSO. The dosed cells are returned to 37ºC and incubated for 24 hours, after which the dose is suctioned off and replaced with media. The plates are then returned to 37ºC for three days. At the end of the three days 10 µL of 0.1% (w/v) alamar blue (resazurin) diluted in phosphate buffered saline is added. The plates are returned to 37ºC to allow the surviving cells to reduce the alamar blue into resorufin. The resorufin fluoresces at 590 nm and this fluorescence is measured usually at 6-8 hours after addition of alamar blue. The fluorescence from the positive control is set at 100% and the fluorescence from the negative control is set at 0 %. The data is fit to a sigmoidal dose response curve and a concentration that yields 50% fluorescence is expressed as the EC50 value.

Results:

As of the last report, one litter of mice had been tested. Two additional litters have been tested. The actual results are shown in Table 1. For each compound tested the TD50 value decreases from wild-type to heterozygous to null. However due to high standard deviations the only statistically significant difference is between WT and null for arsenate (p=0.05).

Table 1. TD_{50} values for Herbimycin A, Cadmium, Geldanamycin, and Arsenate tested on primary mouse embryonic fibroblasts.

Compound	WT	Heterozygous	Null
Herbimycin A nM	676 +/- 594 (n=7)	376 +/- 341 (n=8)	180 +/- 134 (n=6)
$CdCl_2$ µM	4.3 +/- 1.7 (n=7)	4.2 +/- 2.14 (n=8)	3.39 +/- 0.68 (n=6)
Geldanamycin nM	11.1 +/- 5.4 (n=5)	8.11 +/- 2.44 (n=8)	6.16 +/- 3.2 (n=5)
Arsenate µM	199.6 +/- 87 (n=4)	159.9 +/- 97.2 (n=7)	97.5 +/- 32.6 (n=5)

Because of high variability in primary mouse embryonic fibroblasts (MEFs) the data was normalized as follows. For a given litter the average TD50 was calculated regardless of genotype. This average was given a value of 1.00. All values were expressed relative to this average. For example if the average TD50 value for geldanamycin was 10 nM and a null embryo had a TD50 value of 4.5 nM this embryo was listed as having a TD50 of 0.45. The purpose was to remove variability between litters. In the case of each compound tested the TD50 values for null MEFs were below 1.00 and the TD50 values for both wt and heterozygous MEFs were above 1.00 (Table 2).

Table 2. Normalized TD_{50} values for Herbimycin A, Cadmium, Geldanamycin, and Arsenate tested on primary mouse embryonic fibroblasts.

Compound	WT	Heterozygous	Null
Herbimycin A nM	1.44 +/- 0.79 (n=7)	1.20 +/- 0.80 (n=8)	0.53 +/- 0.30 (n=6)
$CdCl_2$ µM	1.00 +/- 0.20 (n=7)	1.09 +/- 0.47 (n=8)	0.86 +/- 0.15 (n=6)
Geldanamycin nM	1.22 +/- 0.53 (n=5)	1.01 +/- 0.27 (n=8)	0.75 +/- 0.45 (n=5)
Arsenate µM	1.16 +/- 0.29 (n=4)	1.13 +/- 0.32 (n=7)	0.68 +/- 0.28 (n=5)

When the data is expressed in this normalized fashion there are more statistical differences (Table 3). Now Herbimycin and Geldanamycin show statistical differences. Cadmium was not significant regardless of how the data was analyzed.

Table 3. Student t-test t values. Cut off values for p = 0.05 are shown in parentheses.

Compound	Normalized WT vs. Null	Normalized WT & HZ vs. Null
Herbimycin A	2.606* (2.179)	2.39* (2.086)
CdCl2	1.659 (2.179)	1.321 (2.086)
Geldanamycin	2.33* (2.262)	1.62 (2.120)
Arsenate	2.40* (2.262)	3.043* (2.120)

From the data, it was determined that cells null for p23 were more susceptible to drugs. With this knowledge in hand, the MOE (molecular organizational environment) program was used to visualize the binding of the mouse Hsp90 homolog (Hsp82) to the mouse homolog of p23 (SBA1) in the attempt to create a peptide that would bind to the p23 and inhibit p23-Hsp90 binding. The crystal structure for Hsp82-SBA1 chaperone complex has been solved ***reference***. This chaperone complex was analyzed and sites of best interaction were isolated based on various computations using the docking feature of MOE. The MOE program had not been previously used; therefore, it took some time to learn the different features of the program and even more time to teach the students involved in the project how to correctly use MOE. From the paper by Ali et al., it was known which parts of Hsp82 interacted with SBA1. The MOE program helped us visualize these interactions sites, and it was found that SBA1 had a concave region that interacted with Hsp82. It was then theorized that the concave region might function as a receptor to a small peptide. Using the MOE program, a small peptide was made whose sequence corresponded to the Hsp82 sequence which binded to the concave region of SBA1. Using the docking feature of MOE, it was found that the peptide interacted with the predicted concave region of SBA1. To ensure that the concave region was a specific binding site, a random peptide was generated and docked with SBA1. Unfortunately, this peptide interacted with SBA1; however, the interaction occurred at a different site. It was anticipated that the region involved in binding would be conserved among various species. A multiple alignment of sequences was

performed with Hsp90 or its homolog from ten various organisms (Fig 1). The proposed sequence for the peptide is highlighted below:

Fig. 1: Multiple alignment sequences of ten organisms

```
FOSB_HUMAN            IRYESLTDPSKLDSGKELHINLIPNKQDRTLTIVDTGIGMTKADLINNLGTIAKSGTKAF 240
FOSB_MOUSE            IRYESLTDPSKLDSGKELHINLIPSKQDRTLTIVDTGIGMTKADLINNLGTIAKSGTKAF 118
FOSB_CHICKEN          IRYESLTDPSKLDSGKDLKINLIPNKHDRTLTIVDTGIGMTKADLVNNLGTIAKSGTKAF 117
FOSB_ZEBRAFISH        IRYESLTDPSKLDSCKDLKIELIPDQKERTLTIIDTGIGMTKADLINNLGTIAKSGTKAF 115
FOSB_BOVINE           IRYESLTDPSKLDSGKELKIDIIPNPQERTLTLVDTGIGMTKADLVNNLGTIAKSGTKAF 113
FOSB_FROG             IRYESLTDPSKLDSGKDLKIDIIPNRLERTLTMIDTGIGMTKADLINNLGTIAKSGTKAF 113
FOSB_DROSOPHILA       IRYESLTDPSKLDSGKELYIKLIPNKTAGTLTIIDTGIGMTKSDLVNNLGTIAKSGTKAF 106
FOSB_FLUKE            IRYKSLTEPSVLDTESELCIKVIPNKADSTLTIIDTGIGMTKADLVNNLGTIARSGTKAF 116
FOSB_ARABISOPSIS      IRFESLTDKSKLDGQPELFIHIIPDKTNNTLTIIDSGIGMTKADLVKNLGTIARSGTKEF 105
FOSB_YEAST            IRYKSLSDPKQLETEPDLFIRITPKPEQKVLEIRDSGIGMTKAELINNLGTIAKSGTKAF 104
                      **::**:: . *:   :* * : *.    .* : *:******::*::******:**** *

FOSB_HUMAN            MEALQAGADISMIGQFGVGFYSAYLVAEKVTVITKHNDDEQYAWESSAGGSFTVRTDT-G 299
FOSB_MOUSE            MEALQAGADISMIGQFGVGFYSAYLVAEKVTVITKHNDDEQYAWESSAGGSFTVRTDT-G 177
FOSB_CHICKEN          MEALQAGADISMIGQFGVGSYSAYLVAEKVTVITKHNDDEQYAWESSAGGSFTVRLDN-G 176
FOSB_ZEBRAFISH        MEALQAGADISMIGQFGVGFYSAYLVAEKVTVITKHNDDEQYIWESAAGGSFTVKPDF-G 174
FOSB_BOVINE           MEALQAGADISMIGQFGVGFYSAYLVAEKVVVITKHNDDEQYAWESSAGGSFTVRADH-G 172
FOSB_FROG             MEALQAGADISMIGQFGVGFYSAYLVAEKVVVITKHNDDEQYAWESSAGGSFTVKVDT-G 172
FOSB_DROSOPHILA       MEALQAGADISMIGQFGVGFYSAYLVADKVTVTSKNNDDEQYIWESSAGGSFTVRADN-S 165
FOSB_FLUKE            MEALNAGADISMIGQFGVGFYSAYLVADKVQVISKNNDDEQYLWESSAGGSFTIRPCS-E 175
FOSB_ARABISOPSIS      MEALAAGADVSMIGQFGVGFYSAYLVADKVVVTTKHNDDEQYVWESQAGGSFTVTRDTSG 165
FOSB_YEAST            MEALSAGADVSMIGQFGVGFYSLFLVADRVQVISKSNDDEQYIWESNAGGSFTVTLDEVN 164
                      **** ****:********* ** :***::* * :* ****** *** ******:
```

As shown, the proposed sequence is almost identical in all organisms with the exception of two amino acids. This supports the idea that this region is important in binding. The peptide will be synthesized using the highlighted sequence from the Hsp82: FMEALSAGADV.

After the sequence for the peptide was determined,

The peptide will be synthesized as a fusion protein. Using the pET42 vector from Novagen the DNA sequence coding the peptide will be cloned into the vector using restriction enzymes PshAI and NcoI. This will allow the expression of a fusion protein (peptide fused to GST Tag) that can be purified with a glutathione column. The peptide will then be released from the Vector sequence using factor X_a.

The assay for binding will carried out as outlined (Cox and Miller). SBA1 with a 6-His tag will be expressed in E. coli, purified on a His-Bind resin, and then dialyzed to remove imidizole. Wild-type Hsp82 will also be expressed in E. coli. An aliquot of Hsp82 cell lysate will be incubated with purified SBA1in the presence of ATP-γ-S. This mixture will be added to His-Bind resin, incubated, washed and pelleted. The pellet will contain the His-Bind resin and any proteins that bind to it, either directly (SBA1) or indirectly (Hsp82). The pellet will be boiled with SDS-PAGE sample buffer, and the supernatant loaded onto SDS PAGE gels. Appearance of Hsp82 on the gel will be indicative of SBA1-Hsp82 binding. If the peptide is added to the mixture and interferes with SBA1-Hsp82 binding, then a lower amount of Hsp82 will be seen on the gel. Mutant Hsp82 will be used as a negative control for the binding.

Vectors containing SBA1 with a 6-his tag, wild-type Hsp82, and mutant Hsp82 were provided by Charles Miller. We have worked out conditions for the expression and partial purification of SBA1. We have the Hsp82 lysate, ran a SDS page of the lysate but saw no difference between induced and uninduced Hsp82. However, even if the protein is expressed, one cannot always see a difference on the gel. Therefore, we cannot be certain that we have expression of Hsp82. ATP-γ-S is on order from Sigma and when it arrives the assay will be run to determine if there is

binding between Hsp82 and SBA1. If no binding is observed, the EnzChek phosphate assay kit for Molecular Probes will be used to determine if Hsp82 is expressed.

Junior Faculty Project #2

Dr. Syed Muniruzzaman, Xavier Biology, has developed a project with Thomas Wiese, Xavier Pharmacy.

Evaluation of Rare Carbohydrates as Inhibitors of Various Biological Processes

On the basis of availability we can classify monosaccharide into two groups, natural and rare. Rare carbohydrates are not abundant in the nature and difficult to produce by organic or chemical reactions. The practical application or usefulness of rare carbohydrates has not been well investigated because of high costs and unavailability. Despite their costs, these rare carbohydrates are very important since they have the potential for use in many areas. For instance, a rare keto hexose, D-tagatose, is now attracting much attention as a low-calorie carbohydrate sweetener and bulking agent (1, 2). D-psicose, a rare keto hexose, does not provide any energy and has other beneficial clinical effects when given to animal orally (3, 4). Recently, it has been reported that a rare aldohexose, D-allose, substantially inhibits segmented neutrophil production and lowers platelet counts without any detrimental clinical effect, might be used in the treatment of myeloid leukemia (5, 6). Another study reported that D-allose inhibited human ovarian carcinoma cells in vitro (7). In February of 2008 Yamaguchi et al reported that D-allose significantly up-regulated thioredoxin interacting protein (TXNIP) gene expression and subsequent G1 cell cycle arrest in hepatocellular carcinoma cells by stabilization of p27kip1(8).

In a previous study Muniruzzaman et al found that rare ketoses, L-fructose and L-xylulose are potential inhibitors of glycoprotein processing enzyme in cell culture system (9). Also, the use of some derivatives of rare carbohydrates as a potent antiviral agent against hepatitis B virus and human immunodeficiency virus have been reported (10, 11). Some other derivatives are also reported as anti-tumor agent for example, bleomysin, which is active against several murine tumors thereby making it useful for cancer treatment (12). Rare sugars are also important as the building block for the synthesis of L-oligo nucleotides and enantio DNA (DNA having L sugar), which are valuable tools for studying protein DNA interactions and are promising antisense agents (13). Utilization of rare sugars for various other purposes still awaits exploration.

The goal of this study is to explore the effects of rare hexoses, pentoses and their deoxy-derivatives on the cancer cell lines. However, initially this study would like to see the effect of only rare ketohexoses on breast cancer cell lines. Amongst the eight ketohexoses L-Fructose, D-Psicose, L-Psicose, D-Sorbose, D-Tagatose and L-Tagatose are rare. These rare ketohexoses will be studied in appropriate cell lines to see any inhibitory or cytotoxic effect.

The effects of the rare sugars will be examined using cell culture bioassays that are routinely used in the Wiese lab that evaluate breast cancer cell proliferation/survival and estrogen reporter gene activity. Our hypothesis is that some of the rare sugars will reduce breast cancer cell proliferation and/or estrogen reporter gene activity with low, general cytotoxicity.

d. Junior Faculty collect preliminary data (Months 7−36).

Year Four Progress (April 19, 2007-April 18, 2008)
See Task 2 c for current status of the Wolfgang-Miller and Muniruzzaman-Wiese projects.

e. Junior Faculty develop grant proposal (Months 36−48).

Year Four Progress (April 19, 2007-April 18, 2008)
No Progress in this area in Y4.

Task 3
Establish infrastructure that will create an environment that fosters breast cancer research, in which Xavier faculty will receive substantive training and become more competitive for DoD funding

Background and Year Four Progress (April 19, 2007-April 18, 2008)
When Xavier was awarded the DOD Breast Cancer grant in April 2004, Dr. Rosenzweig, the project PI, announced that she would leave Xavier in May 2004. A plan was formulated where Dr. Wiese, PI of one of the research projects in the Breast Cancer training program would take over program PI responsibilities along with his research project. He would be provided release time for both tasks and be assisted by a part time administrative assistant that would be hired. Dr Wiese served 5 years as a joint faculty between Tulane and Xavier before moving full time to Xavier in 2003. While at Tulane, he became a member of the Tulane Cancer Center and developed a good working relationship with Dr. Steven Hill, Tulane coPI of this project. Dr. Wiese also had also developed a good working relationship with Dr. Klassen, Xavier Chemistry Department, coPI of the XU-YU DOD Prostate Cancer training program, when Dr. Klassen utilized the cell culture facilities in the Wiese lab in 2003-2004.

Unfortunately, Dr. Klassen elected not to return to Xavier after Katrina. Dr. Wiese, the PI of the Xavier DOD BC program has been asked by the Xavier administration to replace Dr. Klassen as PI of the Xavier DOD Prostate program effective Feb 2006.

The Xavier DOD BC and PC programs continue to operate in parallel with meetings, seminars and discussion sessions involving both groups. In Y1, we established an email list serve for all Xavier and Tulane faculty involved in both XU DOD cancer training projects and this mechanism has been very helpful for rapid communication of cancer center events, project meetings and organizing car pools to LCRC seminars.

It should be noted that the Tulane Cancer Center is part of the Louisiana Cancer Research Consortium (LCRC) that includes the LSU Cancer Center. The LCRC was devised in 2002, involves significant funding from the state of Louisiana and will eventually be housed in a new building between the Tulane and LSU medical centers in New Orleans. The LCRC is co-directed by Dr. Prescott Deininger (Director of Tulane Cancer Center) and Dr. Augusto Achoa (Director of the LSU Cancer Center). Drs. Klassen and Wiese were invited to the first annual LCRC retreat in January 05. The planning process and meetings that took place at this retreat clearly stated that all Xavier faculty interested in or doing cancer research were welcome to participate in the LCRC through adjunct appointments in Tulane or LSU departments. In addition, Dr. Roy Weiner (previous TCC director) has keep in close contact with Dr. Wiese regarding the Xavier DOD Breast Cancer training program and has made it clear that he is

personally committed to helping Xavier faculty develop cancer research projects and programs. He has opened up all the resources of the Tulane Cancer Center core facilities to Xavier researchers and has invited Xavier faculty to be involved in the Tulane Cancer Centers cancer research symposia held each fall. This Mauvernay Research Excellence Award program includes seminars and posters related to cancer research and concludes with a dinner where TCC faculty meet the invited speakers. Several of the XU faculty involved in the DOD cancer training programs attended the Mauvernay Research Excellence Award program in fall 2006 and Drs. Hill and Weiner made a special effort to introduce the XU faculty to TCC faculty and to the invited speakers. Dr. Weiner also has included clinical faculty from the Xavier College of Pharmacy in ongoing initiatives at the Tulane Cancer Center.

One result of this close relationship between Drs. Weiner and Hill of the TCC and Xavier University is the submission of a joint P20 planning grant to the NCI in February of 2005 with Xavier University of Louisiana. This grant was specifically designed to plan long term collaborations between cancer centers and minority serving institutions. Through a series of meetings starting in October 2004, a P20 grant was developed between the Tulane Cancer Center and Xavier University with Dr. Weiner as the Tulane PI and Dr. Kathleen Kennedy, Associate Dean, Xavier College of Pharmacy as the Xavier PI. At the same time, the PI and co-PI of the Xavier DOD Breast Cancer Training Program, Drs. Wiese and Hill became the P20 grant program managers for each respective institution. Drs Wiese and Hill also took responsibility for the majority of the organization, planning and preparation of this panning grant over a 5 month period leading up to submission in February 2005. This NCI P20 program grant was awarded in August 2005 with a start date of October 1, 2005 (during the Katrina evacuation). The good working relationship of Dr. Wiese and Hill, developed largely from the DOD Breast Cancer Training Program and other prior activities, was critical to working out the complex details of this P20 proposal that involved two very different universities. **We feel that the DOD cancer training programs between Tulane and Xavier provided the critical mass required to put together this P20 grant and that the combination of these programs will contribute significantly to the development of self sustaining cancer research programs at Xavier in the future.**

The review of the original Xavier DOD Breast Cancer Training program requested that an administrative assistant be hired to assist the PI in grant management tasks as well as in planning meetings and coordinating communication between all those involved at XU and TU. In August 2004, Mr. Sergio Alcantera was hired as a part time program manager for this project. See Y1 progress report for more details. Mr. Alcantera moved his family to California after Katrina leaving this position open.

With the award of the NCI P20 training grant in 2005, Xavier now had two program grants that had openings a program assistant. With the help of Dr. Roy Weiner at the Tulane Cancer Center and a search process at Xavier, a suitable candidate was identified in early 2006. Ms. Stephanie Colbert was hired by Xavier in February 2006 to support both the DOD BC program and the NCI P20 grant working under the supervision of Dr. Wiese, PI of the DOD BC program and manager of the NCI P20 program.

In the fall of 2007, Xavier University of Louisiana was brought into the Louisiana Cancer Research Consortium (LCRC) to joint Tulane and LSU in the development of a Louisiana Cancer Center that will eventually achieve NCI designation. The LCRC was established in 2002 with state tobacco tax funds and it supports ~$10,000,000 of cancer research and smoking

cessation activates each year. While all cancer researchers at Xavier had been members of the LCRC (through involvement in DOD programs and membership in the Tulane Cancer Center), this new status brings LCRC resources directly to Xavier. In the 2007-2008 state fiscal year, Xavier received to develop LCRC activities and starting in the 2008-2009 fiscal year, Xavier will receive each year to develop and maintain cancer research programs. **It is important to recognize that Xavier involvement in the LCRC came about only because of Xavier involvement in the DOD Cancer Training programs.**

The logical choice for the leadership for the Xavier LCRC participation was Xavier faculty already involved in leading cancer programs funded by the DOD and NCI. Dr. Wiese was established as the LCRC Associate Director for Xavier and Ms. Colbert as the Xavier LCRC program assistant. Dr. Wiese now attends twice monthly LCRC leadership meetings with the co-Directors, CEO and Administrator for Research as well as the every other month LCRC board meetings and Finance meetings. He also is a member of the LCRC Scientific Executive Committee that meets monthly to deal with review of LCRC supported internal funding requests, review and development of core facility operations and LCRC long term planning. Ms. Colbert attends the LCRC business manager, finance and board meetings in addition to working with the LCRC staff on public relations, annual reports, news letters and some fund raising organization.

The first task of the Xavier LCRC was to plan goals and structure at Xavier. An internal advisory board was established consisting of the chairs of the Biology, Chemistry, Division of Basic Pharmaceutical Sciences, Division of Clinical and Administrative Sciences, the Director of the Xavier Center for Health Disparities Research and Education and the Senior VP for Resource Development. This XU LCRC IAB meets every two months to advise and over see the activities of the XU LCRC Associate Director. In addition, the IAB reviews submissions requests for XU LCRC funding (pilot grants, seed grants, instrument requests, etc) and serves as a direct line of communication to and from university units with the bulk of the cancer researchers on campus. While XU LCRC IAB represented units are looking to recruit faculty, the XU LCRC will with them to hire and support cancer research focused faculty. In 2007-2008, some cancer research faculty just hired were supported with small startups. For 2008-2009, we are planning coordinated advertisement and hire of up to 6 cancer research focused faculty that will receive three years of significant startup funding to establish their research at Xavier.

A plan was developed where the bulk of the XU LCRC funding would go to new hire start up packages (cancer research only) while cancer research core infrastructure, pilot and seed grants, development of core facilities and cancer research seminars and research discussion meetings would also be supported. **Xavier involvement in the LCRC has provided the ability to recruit cancer research focused faculty (startups) and to sustain cancer research activities developed by the XU DOD Cancer training programs (seminars, research discussion meetings, development of new projects, research infrastructure).**

The XU LCRC budgets for FY08 and 09 are included in Appendix 1

With Xavier a member of the LCRC, Dr. Wiese the Associate LCRC Director for Xavier, and Xavier President Dr. Norman Francis a member of the LCRC board, Dr. Wiese is in close contact with Dr. Francis as well as senior LCRC administrators about the development of cancer programs at Xavier.

a. Grant membership in the Tulane Cancer Center to Xavier researchers. Drs. Wang and Wiese will be granted a status of contributing members and the junior faculty will be granted a status of associate members. Please see attached TCC publication for the definitions (Month 1).

Year Four Progress (April 19, 2007-April 18, 2008)
All 4 Xavier faculty involved in this program have either been approved as adjunct faculty at Tulane or this approval is pending. Once approved, this status allows Xavier faculty to be contributing members of the Tulane Cancer Center (TCC) as well as the Louisiana Cancer Research Consortium (LCRC). As members, these faculty can use the various core facilities at the cancer center at a reduce rate. To date, all Tulane mentors have facilitated the use of any needed cancer center cores with or with out membership. This adjunct status also allows the Xavier faculty doing cancer research to use the Tulane library resources.

b. Include Xavier researchers in Tulane Breast Cancer focus group and Journal Club (Months 2).

Year Four Progress (April 19, 2007-April 18, 2008)
All faculty involved in the Xavier DOD Cancer programs are now integrated into the Molecular Signaling focal group of the LCRC. These faculty are exposed to a wide range of cancer research by attending the Molecular Signaling research meetings. In addition, these faculty are now involved in building stronger ties between Xavier and the LCRC. We have established a monthly Cancer Research lunch meeting at Xavier (see Task 3 e below).

It should be noted that Xavier cancer research faculty are informed about and encouraged to attend all the cancer related seminars and working groups in the LCRC. The LCRC invited speaker series is every other Thursday at noon alternating between Tulane and LSU. Other LCRC discussion groups include: Friday Afternoon Encounters to discuss recent data, the Immunology Club, the Apoptosis and Cell Survival meetings (2x per month) and the weekly Prostate Cancer Group Meetings at LSU; the weekly Breast/Ovarian Group Meeting and the weekly Stem Cells and Cancer Group Meeting at Tulane Cancer Center. The two DOD programs at Xavier have also established a bi-weekly Cancer research Discussion group where faculty involved in DOD projects rotate presenting about the latest status of their project. Tulane mentors and collaborators as well as students are invited to these meetings and this program has been very helpful in bringing our group together. This cancer focused work in progress series has become very popular among faculty interested in cancer research and new faculty hired to do cancer research supported by the LCRC.

c. Grant access to core research facilities at Tulane Cancer Center (Month 1).

Year Four Progress (April 19, 2007-April 18, 2008)
Access to TCC and LCRC core facilities has been granted to Xavier faculty. These cores include: Genomics, Proteomics, Biostatistics/Bioinformatics, Immunology, and Tissue Acquisition. To date, no Xavier faculty have required the use of these facilities.

d. Include a student in each research project (Month 2 for Drs. Wang and Wiese and Month 8 for the junior faculty).

Year Four Progress (April 19, 2007-April 18, 2008)
See Task 1 above.

e. Establish a monthly brown−bag lunch meeting to bring up research related issues, review proposals and papers, or brainstorm on new directions to improve the cancer program (Month 1).

Year Four Progress (April 19, 2007-April 18, 2008)
We have established a monthly Cancer Research lunch meeting at Xavier where the faculty involved in our DOD Cancer programs rotate in giving "work in progress" presentations to the group. These meetings are held on a Monday at noon each month and are well attended by all members of the labs involved as well as our Tulane mentors-collaborators. These meetings have not only assisted Xavier faculty with their projects, but have also provided a place where we can all se what each other are doing. Other Xavier faculty interested in cancer research are now attending these meetings and we may expand these sessions to twice a month. The schedule for these meetings is listed in Appendix 3.

e. Hold an annual workshop, open to all in the Xavier and Tulane communities and Xavier student body, for all BC participants to present results of the preceding year. Faculty, students, and staff will attend and at least one person from each group will present a talk; students will present posters (Months 12, 24, 36, 48).

A. First workshop titled "Molecular Signaling in Breast Cancer" (Month 12).
B. Second workshop titled "Breast Cancer and the African American Community" (Month 24).
C. Third workshop titled "Funding Opportunities in Breast Cancer Research" (Month 36).
D. Forth workshop titled "Drug Design and Delivery in Breast Cancer" (Month 48).

Year Four Progress (April 19, 2007-April 18, 2008)
Our attempts to hold a Cancer Research symposia at Xavier have been foiled by scheduling conflicts with university and department events. We will continue trying to developed a seminar series at Xavier as well as a mini-symposia at with poster sessions. It should be noted that all Xavier students involves in the DOD programs at Xavier present at the universities annual Festival of Scholars in April. With Xavier now part of the Louisiana Cancer Research Consortium (LCRC), Xavier cancer research faculty will be presenting at the LCRC annual retreat held each spring. **Participation in this city wide symposia involving cancer researchers from Tulane and LSU along with Xavier achieves our goal of getting Xavier faculty involved in an annual cancer focused symposia.**

f. Subscribe to breast cancer related journals (Month 1).

Year Four Progress (April 19, 2007-April 18, 2008)s
In Y1, we purchased a subscription to the online journal Breast Cancer Research. In Y2, we determined that we could get access to the journal Proteomics through the Xavier Library and that a subscription was not needed. Access to Tulane library resources is still limited for some XU faculty. Only faculty with adjunct appointments have off campus online access. Dr. Wiese has access (from prior adjunct appointment) and is serving as the access point for journal articles needed from Tulane. In Y3 we must established XU faculty as adjuncts at Tulane to resolve this problem. We now maintain these subscriptions and cancer research faculty at Xavier have access to the journals at the Tulane Library.

Key Research Accomplishments

- Comparative proteomics was carried out on the two breast cancer cell lines, MCF-7-MEK5 and MCF-7-VEC. As described earlier, MCF-7-MEK5 is a TNF-α-resistant breast cancer cell line derived from the wild-type, i.e. TNF-α-sensitive MCF-7 cells. MCF-7-MEK5 is characterized by 1) a morphological change consistent with epithelial-to-mesenchymal transition, 2) over expression of MEK5 (MAPK), 3) resistant to tumor necrosis factor-α and tamoxifen, and 4) estrogen independent. MCF-7-VEC represents a control cell line transfected with empty vector.
- We have identified seven protein spots that are differentially expressed in MCF-7-MEK5 and MCF-7-VEC using 2-D gel electrophoresis. Real time PCR analysis confirmed the findings in proteomics work.
- We have obtained evidence at both the gene and protein expression level, that MCF-7-MEK5 cells express EMT markers, suggesting involvement of MEK5 in the regulation of EMT in breast cancer cells.
- A proposal for the DoD IDEA AWARD (oppW81XWH-08-BCRP-IDEA) is in preparation for submission in May, 2008. The title: MEK5-Erk5 Signaling Regulates Epithelial-to-mesenchymal Transition in Breast Cancer Progression.
- PCR Array gene array methods have been established and are now being applied to the issue of pesticide exposure and breast cancer progression.
- A new project continues to study the role of P23 in HSP90 control of cell signaling.
- A new project has been developed to examine rare sugars as potential cancer therapy agents.
- All members of this research team are either now, or in the process of being appointed as adjunct faculty at Tulane.
- XU is now a member of the Louisiana Cancer Research Consortium (LCRC) and has an annual budget with funds to continue faculty research development activities started by this DOD program. This includes recruitment of cancer research faculty supported by start up funds, the development of cancer research infrastructure and cores and the support for pilot project and other support for Xavier faculty.

Reportable Outcomes

A manuscript based on these findings has been submitted to the *Journal of Breast Cancer Research* in March of 2008:

Proteomic analysis of tumor necrosis factor-α resistant human breast cancer cells reveals a MEK5/Erk5-mediated epithelial-mesenchymal transition phenotype

Changhua Zhou, Ashley M Nitschke, Wei Xiong, Qiang Zhang, Yan Tang, Michael Bloch, Steven Elliott, Yun Zhu, Lindsey Bazzone, David Yu, Christopher B Weldon, Rachel Schiff, John A McLachlan, Barbara S Beckman, Thomas E Wiese, Kenneth P Nephew, Bin Shan, Matthew E Burow, Guangdi Wang

Conclusion

We have established three collaborative breast cancer research projects and are in the process of building one more new project. We have built a framework of activities for XU faculty to utilize for interaction with the TCC/LCRC to develop cancer research initiatives involving Xavier undergraduate and pharmacy students. Most importantly, our program team has reestablished their labs, validated project data obtained before the Katrina shutdown and the projects are now moving forward on new aims. With Xavier a member of the LCRC, we will continue supporting

the development of cancer research on campus through variety of mechanisms.

Year Five Synergy and Opportunities

In Y5, we plan to build on established interactions with the XU-TU DOD Prostate Cancer program and the new involvement in the LCRC. The main goal of Y5 will be continue on program and project aims now that our labs have been re-established and to continue addressing project aims. We will continue building our small cancer research network at Xavier by holding regular cancer research discussion meetings, developing the aims of the NCI P20 grant funded in 2005 and our participation in the LCRC. Our long term goal is to establish a core of faculty at Xavier that are active in cancer research and education.

Year Five Challenges

The main obstacle is the increased administrative loads of participating faculty. Dr. Wiese, PI of both the Xavier DOD Breast and Prostate programs is also the manager of the Xavier NCI P20 program and now Associate Director of the LCRC for Xavier where he is directing the development of new cancer focused programs at Xavier including recruitment and start up packages for 6 new faculty and the development of core support resources.

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Appendices

Appendix 1

Xavier LCRC 2007-2008 Budget (Year 1: July 1, 2007-June 30, 2008)

			Year 1
A.		**Scientific Program Development - Faculty Recruitment**	
1		**Individual Faculty Recruitment Packages and Related Expenses**	
	1a	Faculty Recruitment for Fall 2008	$8,000
B.		**Scientific Program - Other**	
2		**Program Enhancement**	
	2a	Scientific Seminars and Speakers (Travel and Honorarium)	
		2-3 Invited Speakers	$4,600
	2b	Cancer Research Discussion meetings at Xavier University	
		15 lunches for 25 people	$6,400
	2c	IAC meetings - 10 lunches for 8 people each	$1,200
	2d	Parking for Associate Director and Program Assistant	$144
	2e	NCI Fellows/MARC/RISE meetings - 20 lunches for 20 people	$4,000
	2f	Scientific Program and Grant Consultants	$3,000
	2g	Travel Budgets	
		Travel and Training for XU faculty, scientific meetings	$5,285
	2h	Cancer Research Infrastructure: Key Instrumentation	$85,105
	2i	Cancer Reseach Infrastructure: Research Personnel Molecular Biology	
		Salary and Fringe for: Elena Skripnokova	$22,125
3		**Faculty Research Development**	
	3a	Start up Funds, start date 9/1/07	
		Gloria MaGee, Ph.D.	$25,000
		KaTani Parker-Johnson, Ph.D.	$25,000
		Karen Zhang, Ph.D.	$17,000
	3b	Seed Funds	
		Robert Blake, Ph.D.	$24,780
		Maryam Foroozesh, Ph.D.	$46,775
	3c	Bridge Funds	
		Gurdial Arora, Ph.D. (Summer Salary $6889 salary/$1240 fringe)	$8,129
		Staff Bridge Support: Dr. Guo (2 month) and Dr. Zhu (3 month)	$17,115
	3d	NCI Incentive Program	n/a
	3e	Competitive Advantage Fund	n/a
4		**Institution Specific Program Needs for Consortium Development**	
	4a	Office Supplies and Program Support	$3,826
	4b	XU LCRC Office Equipment, computers, furniture	$7,500
5		**Renovation of Existing Space: Core Facility Development**	n/a
6		**Program Leader Salary Support**	n/a
7		**Program staff support for scientific leadership**	
	7a	Stephanie Colbert, 25%. Salary requested from LCRC = $10599 fringe = $1908	$12,507
8		**Clinical Trials and Translational Research**	n/a
9		**Clinical Trials System Support**	n/a
10		**Clinical Research Fellow Support**	n/a
C.		**Core Operations**	
11		**Core Operations: Planning Development of XU Core Facilities**	
	11a	Core Personnel	
		Core Faculty Support: 2 people for 2 months at $45K per year	$17,700
		Proteomics Core - G. Wang, 10% in kind	$0
		Drug Structure and Modeling Core - C. Stevens, 10% in kind	$0
	11b	Core Equipment/Software	
		Proteomics	$71,130
		Drug Structure and Modeling	$71,130
	11c	Tissue-Serum Banking	n/a
	11d	Biostatistics/Bioinformatics Core Development	n/a
D.		**Scientific Administration**	
12		**Co-Directors, Deputy Directors, Associate Directors Salaries**	
	12a	Thomas Wiese, 10%. Salary requested from LCRC = $8516. Fringe = $1533	$10,049
13		**Admin and Business Support Staff for Co-Directors, Deputy and Associate Directors**	n/a
14		**Grant Administration**	n/a
15		**The Consortium Scientific Planning Retreat**	n/a
16		**Consortium Consultant**	n/a
17		**Consortium Scientific Administration Travel**	
	17a	Travel for Associate Director, Dr. Thomas Wiese	$2,500
18		**Consortium Scientific Public Relations**	n/a
19		**Executive Director for Scientific Programs**	n/a
20		**External Scientific Advisory Board**	n/a
		Total	$500,000

Original FY09 Budget

Xavier LCRC 2007-2008 Budget (July 1, 2008-June 30, 2009)

			FY09
A.	**Scientific Program Development - Faculty Recruitment. SPD1**		
1	**Individual Faculty Recruitment Packages and Related Expenses**		
	1a	Faculty Recruitment Start up Funds	
		College of Arts and Sciences Faculty (2) ($75,000 each)	$150,000
		College of Pharmacy Faculty (1) ($100,000)	$100,000
B.	**Scientific Program - Other. SPD2**		
2	**Program Enhancement**		
	2a	Scientific Seminars and Speakers ($2000 each for Travel; $1000 each for Honorarium)	
		5 Invited Speakers	$15,000
	2b	Cancer Research Discussion meetings at Xavier University	
		16 lunches for 25 people	$6,800
	2c	IAC meetings - 9 lunches for 8 people each	$1,200
	2d	off campus Parking for Associate Director and Program Assistant	$200
	2e	NCI Fellows/MARC/RISE meetings - 20 lunches for 20 people	$4,000
	2f	Scientific Program Consultants	$3,000
	2g	Travel Budgets	
		Travel for 4 XU faculty to attend scientific meetings	$10,000
	2h	Cancer Research Infrastructure: Key Instrumentation Support	$53,020
	2i	Cancer Research Infrastructure: Molecular Biology Specalist	
		Salary and fringe: Elena Skripnokova ($46,350 salary/$8,343 frin	$54,693
	2j	Cancer Research Infrastructure: Proteomics Specalist	
		Salary and fringe: Peng Ma ($43,000 salary/$7740 fringe)	$50,740
	2k	Xavier Student Cancer Training and Education	
		includes Colligiate Cancer Council, New Student Trainees (2)	$20,500
3	**Faculty Research Development**		
	3b	Seed Funds (2 projects continued from year one -	
		12 months for Dr. Blake and Dr. Foroozesh)	$131,110
		Seed Funds (1-2 new seed projects starting 9/1/08)	$110,000
	3c	Bridge Funds	n/a
	3d	NCI Incentive Program	n/a
	3e	Competitive Advantage Fund	n/a
4	**Institution Specific Program Needs for Consortium Development**		
	4a	Office Supplies and Program Support	$2,000
	4b	XU LCRC Office Equipment, computers, furniture	$2,000
5	**Renovation of Existing Space: Core Facility Development**		
	5a	Core Facility Development	n/a
6	**Program Leader Salary Support**		n/a
7	**Program staff support for scientific leadership**		
8	**Clinical Trials and Translational Research**		n/a
9	**Clinical Trials System Support**		n/a
10	**Clinical Research Fellow Support**		n/a
C.	**Core Operations. CO-839**		
11	**Core Operations: Planning Development of XU Core Facilities**		
	11a	Core Personnel	
		Drug Design Core - C. Stevens, 10% ($8420 salary/$1516 fringe)	$9,936
		2 Research faculty in Drug Design (2 x $45,000 salary/$8100 fringe)	$106,200
	11b	Core Equipment/Software/supplies/maintenance/service	$25,000
	11c	Tissue-Serum Banking	n/a
	11d	Biostatistics/Bioinformatics Core Development	n/a
D.	**Scientific Administration. SA-839**		
12	**Co-Directors, Deputy Directors, Associate Directors Salaries**		
	12a	Thomas Wiese, 20%. Salary requested from LCRC = $17,884 Fringe = $3219	$21,103
13	**Admin and Business Support Staff for Co-Directors, Deputy and Associate Directors**		n/a
	13a	Stephanie Colbert, 20%. Salary requested from LCRC = $8897.50 fringe = $1	$10,499
14	**Grant Administration**		n/a
	14a	Stephanie Colbert, 20%. Salary requested from LCRC = $8897.50 fringe = $1	$10,499
15	**The Consortium Scientific Planning Retreat**		n/a
16	**Consortium Consultant**		n/a
17	**Consortium Scientific Administration Travel**		
	17a	Travel for Associate Director, Dr. Thomas Wiese	$2,500
18	**Consortium Scientific Public Relations**		n/a
19	**Executive Director for Scientific Programs**		n/a
20	**External Scientific Advisory Board**		n/a
		Total	$900,000

Appendix 2

Louisiana Cancer Research Consortium

Cancer Research Discussion Meetings

Noon – 1:00pm
Room 420 College of Pharmacy

Monthly Research Meeting Schedule
Last Monday of the Month – Lunch provided (11:45am)

Date:	**Presenters**
Monday October 29, 2007	Stevens-Jones Project
Monday November 12, 2007	Foroozesh Project
Monday November 26, 2007	Wang-Burow Project
Monday December 10, 2007	Wolfgang-Miller Project
Monday January 14, 2008	Ireland-Mageed Project - CANCELLED
Monday January 28, 2008	Kolesnichenko Project Title: "Novel imaging agents for early cancer diagnostics by MRI"
Monday February 11, 2008	canceled
Monday February 25, 2008	Cancelled due to LCRC Retreat
Monday March 3, 2008	Arora-Sikka Project
Monday March 10, 2008	Parker-Johnson Project
Monday March 24, 2008	Zhang Project
Monday April 14, 2008	MaGee Project
Monday April 28, 2008	Ireland-Mageed Project - CANCELLED
Monday May 12, 2008	Wiese-Hill Project
Monday May 26, 2008	Planning Meeting